Fat and Carbohydrate Metabolism in Humans

A Study of Nutritional and Hormonal Effects

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• As an index to the rate of fat utilization in human subjects, the recovery of all radioactive carbon dioxide in the expired air was measured for one hour following intravenous injection of palmitate-1-C^{14}. In the normal fasted subject, about 10 per cent of the injected dose was recovered, and the proportion was lowered to about 5 per cent by administration of glucose. With prolonged fasting, the recovery of radioactive carbon dioxide did not change, despite a rising concentration of fatty acids in the serum. This was interpreted as due to the development of a balance between increasing mobilization and oxidation and was thought to indicate increasing fatty acid oxidation.

In chronic undernutrition and diabetes mellitus there was increased fatty acid oxidation due presumably to adaptation to a chronic increase in fat utilization for energy.

Administration of human growth hormone did not increase fat oxidation but prevented the usual inhibition produced by glucose. This was interpreted to mean that growth hormone increases fat utilization only indirectly by inhibiting the usual preferential utilization of glucose over fat.

ADVANCES IN KNOWLEDGE and techniques have made it possible to study in some detail the influences of nutritional and hormonal factors on metabolism in humans. New knowledge of the importance and role of fatty acids has provided a tool to study the rate of fat mobilization and utilization. New advances in instrument design have provided sensitive and reliable equipment to detect radioactive carbon dioxide coming from the lungs of normal subjects after injection of safe amounts of radioactive fatty acids. New knowledge of species specificity of growth hormone and new methods of purification have provided purified potent preparations of human growth hormone for experimental use.

In order to understand the basis and significance of the research to be presented, a background of knowledge of modern concepts of fatty acid metabolism is necessary. Long-chain fatty acids of 16 to 20 carbon length are held in fat depots as triglycerides and mobilized into the circulation when needed for energy (Chart 1). These "natural" fatty acids are chiefly oleic, linoleic and palmitic[2] and are carried in the circulation bound to serum albumin. In this form they do not cause lipemia and it is of interest that the so-called "clearing reaction" results from the action of heparin to break down triglycerides in the circulation to fatty acids which are bound by serum albumin.[6]

The fatty acids are found in the serum in low concentrations after meals, varying from 0.3 to 0.5 mEq. per liter (8 to 12 mg. per 100 cc.) and rise gradually with prolonged fasting to 1.5-2.0 mEq. per liter (40 to 50 mg. per 100 cc.). Despite the low concentrations the fatty acids can provide a large number of calories for energy because they so rapidly leave the circulation (half-time about 2 minutes)[7] and are quickly burned to carbon dioxide. After intravenous injection of palmitic acid labeled with carbon[14], radioactive carbon dioxide appears in the expired air within 2 minutes. It has been found that mobilization of fatty acids from the depots into the circulation is influenced by many nutritional and hormonal factors. As described above, as fasting is prolonged, there is a gradual rise in the content of fatty acids in the blood, presumably to provide fat for fuel in greater amounts. This implies that the mixture of foods used by the body with prolonged fasting consists of increasing amounts of fat. Early demonstration of decreasing respiratory quotient with fasting, approaching 0.7 characteristic of fat oxidation, supports this concept.

The availability of carbohydrate is intimately associated with the mobilization of fatty acids, and this may account for the rise that occurs as fasting is prolonged and carbohydrate stores are depleted. In untreated diabetes, a situation in which the cells of the body cannot use glucose, the concentration of fatty acids in the blood is elevated. Following glucose administration, the concentration of fatty acids in the blood falls and remains low for 2 to 3 hours due to inhibition of mobilization of fatty acids from the adipose depots. Administration of insulin results in a prompt decrease in fatty acid concentration (Chart 1).[1] If the blood sugar falls too low, resulting in epinephrine release from the adrenal medulla, there is a sharp but transient rise in blood fatty acids (Chart 1). The concentration may rise

Presented before the Section on Internal Medicine at the 89th Annual Session of the California Medical Association, Los Angeles, February 21 to 24, 1960.

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This work is supported by USPHS grant A-1847.

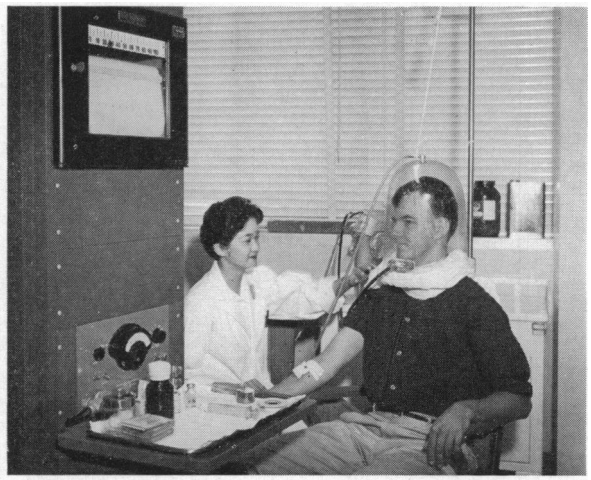

Figure 1.—Apparatus for measurement of radioactive carbon dioxide after intravenous injection of carbon labeled fatty acids.

to several times the starting level but usually falls back by the end of one hour.[5] Epinephrine directly stimulates release of fatty acids from adipose tissue, even *in vitro*, and thus the effect is direct. It is probable that the occasional occurrence of fatty liver, lipemic serum and eruptive xanthomata seen in poorly controlled diabetes results from excessive and prolonged mobilization of fatty acids. These may go to the liver, where they are held in ester form, filling the liver with fat, and be released back into the circulation as triglycerides producing lipemia.

The effects of growth hormone on fatty acid utilization were selected for study since in acromegalic humans and in animals treated with growth hormone there is a change in metabolism. This is characterized by deposition of new protein, enhanced catabolism of fat and depressed utilization of carbohydrate. These studies were undertaken in order to learn more of the mechanism of these changes in metabolism.

In the studies to be described the recovery of total radioactive carbon dioxide in the expired air during the hour following intravenous injection of a tracer dose of labeled fatty acid was used as an index to the rate of fat utilization. The fatty acid used was palmitate labeled with carbon^{14} in the carboxyl position. It was bound to human serum albumin by dissolving 1 millimol palmitate-1-C^{14} acid (Research Specialties Co.) in warm KOH and adding 0.5 millimol Cutter human serum albumin (salt poor). The sterile solution was injected intravenously through an indwelling needle in an antecubital vein from which specimens of blood were obtained for measurement of fatty acid concentration. A helmet was placed over the head of the subject (Figure 1) and a stream of compressed

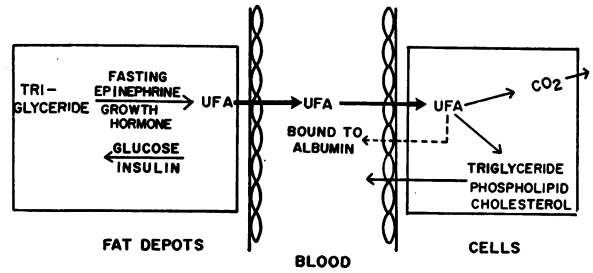

Chart 1.—Scheme of movement of fatty acids (UFA) from fat depots into circulating blood and into liver. Some factors which affect these steps are given.

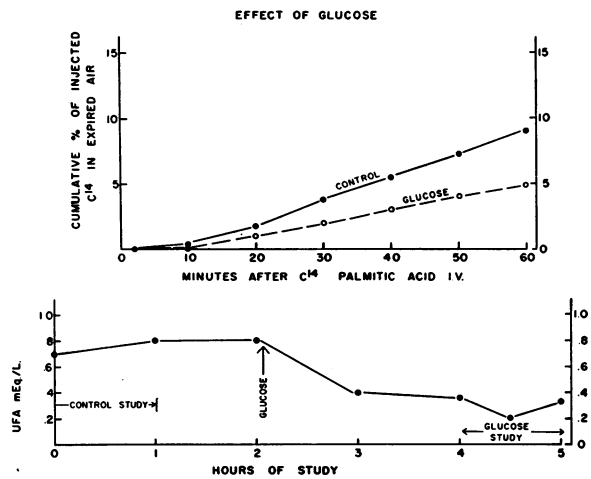

Chart 2.—Shown in the upper part of the chart is the per cent of the injected tracer dose of palmitic acid appearing as carbon dioxide before and after glucose administration. The lower part gives serum fatty acid (UFA) concentration during the period of the entire study.

air carried expired air into an ionization chamber which continuously measured the carbon^{14} content of the chamber. The radioactivity was recorded continuously by a pen writer, making it possible to calculate at any time how much of the administered dose had been oxidized. It was not possible in these experiments to measure the carbon dioxide content of the air in the ionization chamber for calculation of the specific activity of the expired carbon dioxide. Hence these results must be considered to be preliminary.

RESULTS

Normal control and effect of glucose: The cumulative outflow of $C^{14}O_2$ from a normal young subject during the hour after intravenous injection of a tracer of palmitate is shown in Chart 2. The curve labeled *control* was obtained in the morning in the fasted state. The lower curve labeled *glucose* was obtained during the hour beginning 4 hours later and following 2 hours of glucose administration which was continued during the hour of the second

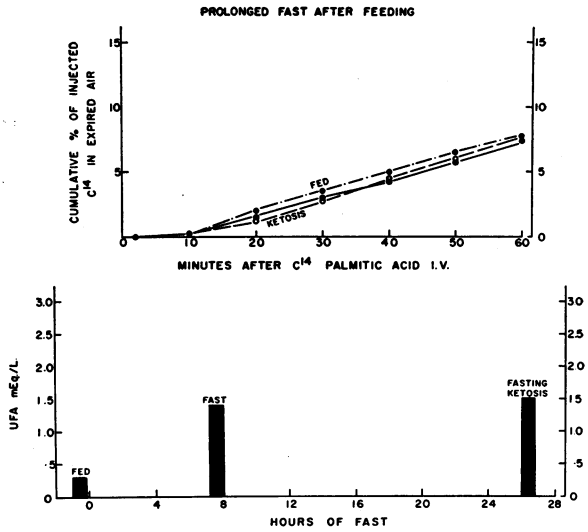

Chart 3.—Results of three studies of fatty acid (UFA) oxidation in a normal subject—the first in the morning after breakfast and glucose, the second in the afternoon and the third the following afternoon.

study. A total of 200 gm. of glucose was given—100 gm. orally and 100 gm. intravenously. These results, which are typical of other similar studies, reveal that glucose administration lowers the outflow of radioactive carbon dioxide to about one-half the control value. The lower part of the chart gives the fatty acid concentration in mEq. per liter of serum. The concentration rose slowly on fasting but decreased sharply following glucose administration.

Prolonged fasting: The results of a series of studies on a normal subject are shown in Chart 3. The first study was made in the morning after breakfast and glucose administration. The subject was then fasted and the second study was conducted in the afternoon. Fasting was continued and the final study took place the following afternoon. By the time of the third study starvation ketosis was present, as manifested by 4+ acetone in the urine. The recovery of radioactive carbon dioxide was the same in all three studies. The bars on the lower part of the chart show the rising concentration of fatty acids in the blood. These results were interpreted to mean that as fasting is prolonged, the pool of fatty acids is enlarging; thus recovery of the same amount of the tracer from a larger pool indicates increasing oxidation of fatty acid. The recovery of the tracer is the same because there is a balance between the increasing pool size and the increasing rate of oxidation of fatty acids.

Chronic undernutrition: The rate of oxidation of the tracer dose of palmitate was studied in four patients who were chronically undernourished (Chart 4). Of these, two had intestinal malabsorption, one diabetes and one cancer. The curves of

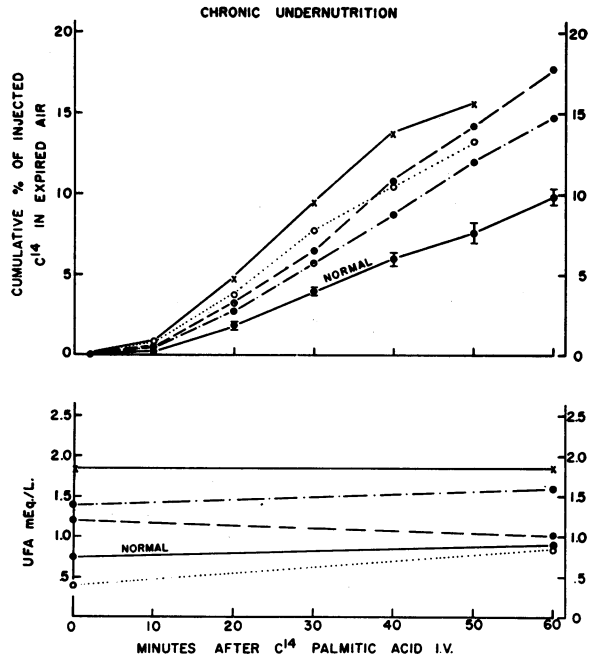

Chart 4.—Fatty acid oxidation studies in four chronically undernourished patients compared with the results in normal subjects. The serum concentration of fatty acids (shown in the lower part of the chart) are mostly above normal.

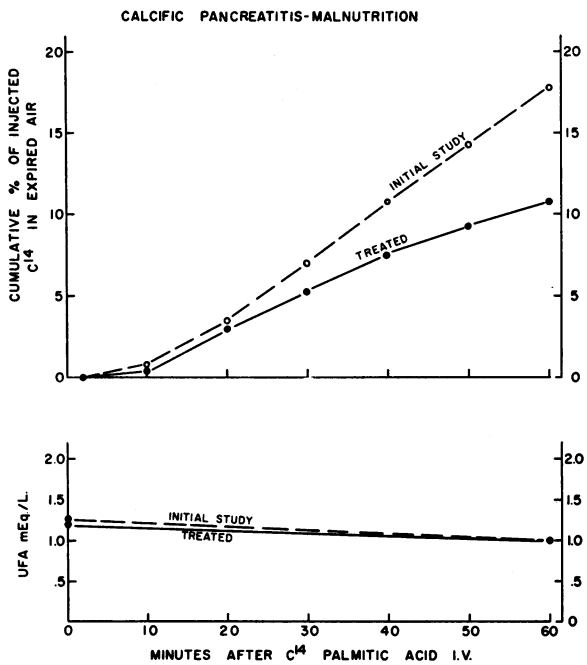

Chart 5.—Results of oxidation studies in a patient with calcific pancreatitis before and nine days after treatment with insulin and pancreatic enzymes. Serum concentrations of fatty acids (lower part of chart) were unchanged.

cumulative recovery of $C^{14}O_2$ are all much higher than the normal curve. Shown in the lower part of the chart is the amount of fatty acid in the blood at the beginning and at the end of each study. In most cases these amounts were above normal. These results were interpreted to indicate that these patients had adapted to chronic undernutrition and the use of adipose stores for fuel, by an increased rate of fat utilization.

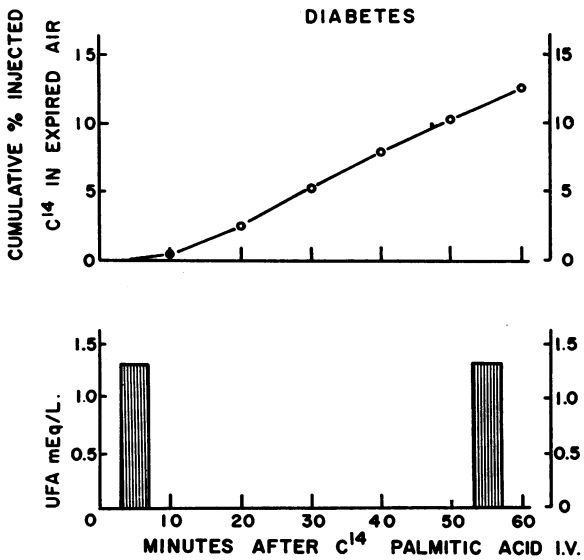

Chart 6.—Average of three patients with diabetes mellitus 24 hours after last dose of insulin. Output of $C^{14}O_2$ (upper) and the serum content of fatty acid (UFA) are above normal.

One of these patients, with malabsorption due to calcific pancreatitis, was restudied after nine days of insulin and pancreatic enzyme therapy. The recovery of $C^{14}O_2$ after treatment (Chart 5) was within normal limits and was not much more than half the amount before treatment. Shown at the bottom of the chart are the blood fatty acid concentrations, which were unchanged.

Diabetes: The proportion of injected $C^{14}O_2$ recovered from expired air of three patients with diabetes mellitus was significantly higher than for a normal subject. Chart 6 shows the average for the three patients. In the lower part of the chart are the average concentrations of the fatty acids in the blood at the beginning and end of the study. These are also higher than normal. These results suggest that patients with diabetes who are poorly controlled will have elevated content of fatty acid in the blood and will oxidize fats at an accelerated rate due to adaptation to fat utilization. Such a conclusion must be tentative in view of the small number of patients and the lack of specific activity measurements.

Effects of growth hormone: An example of results of preliminary studies on the effects of growth hormone on palmitate oxidation is shown in Chart 7. At the upper left part of the chart are the curves of

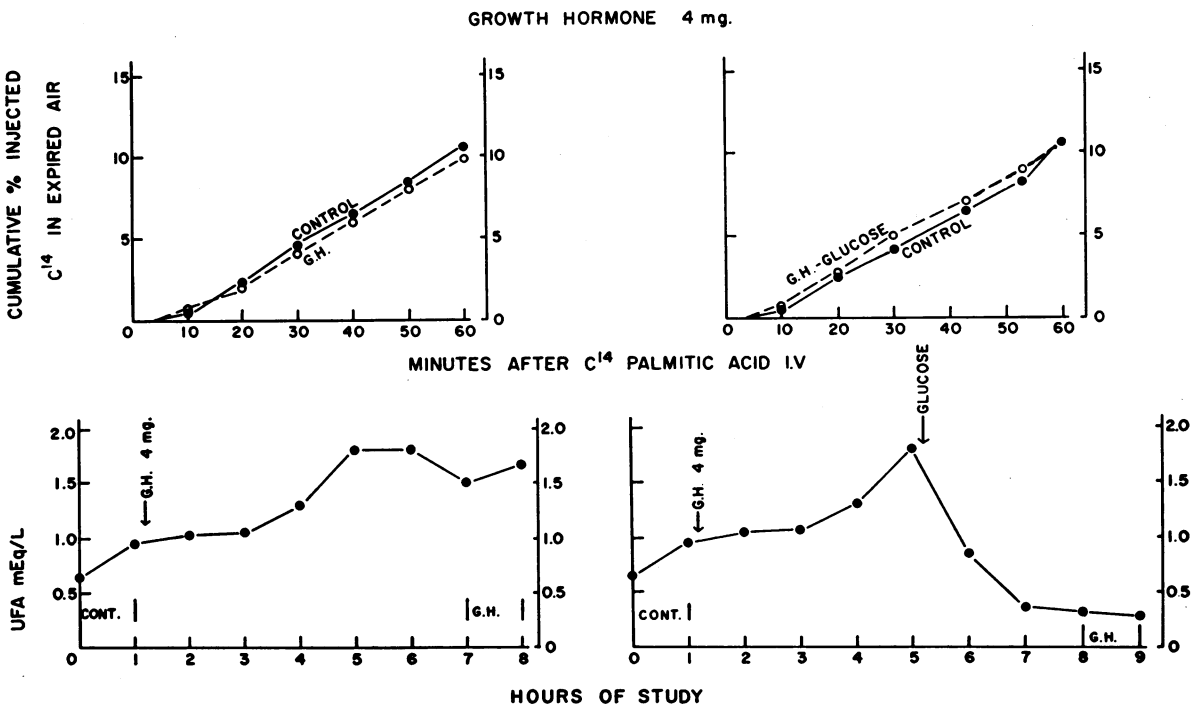

Chart 7.—Effects of human growth hormone on fatty acid oxidation (upper left) and serum concentration (lower left). Effects of growth hormone and glucose (upper right) on fatty acid oxidation and on serum fatty acid concentration (lower right).

outflow of $C^{14}O_2$ before and 7 hours after intramuscular injection of 4 mg. of human growth hormone prepared by Raben.* The curves are not different. Below are shown the fatty acid concentrations in the blood, first the slow rise due to fasting and then the rapid rise due to the fat mobilizing property of growth hormone. The right upper part of the chart shows the result of a similar series of studies with, however, the administration of glucose beginning 3 hours before the second study. In contrast to the usual result of glucose administration (Chart 2), there was no fall in $C^{14}O_2$ recovery from glucose after growth hormone administration. The lower part of Chart 7 shows the usual decrease in the blood content of fatty acid which follows glucose administration. These results were interpreted to mean that growth hormone does not directly increase fatty acid oxidation but increases fat utilization by inhibiting the usual preferential utilization of glucose.

DISCUSSION

These preliminary studies suggest that this technique for measuring the rate of fat utilization in humans yields valid information. The inhibition of fat oxidation by glucose confirms earlier work in rats,[8] dogs[4] and humans.[3] Thus it appears that the organism subsisting on a mixed diet will preferentially use carbohydrate, and that administration of carbohydrate inhibits both the mobilization of fatty acids from depots into the blood (thus lowering the amount of these acids in the blood) and the oxidation of fatty acids by the peripheral tissues. The inhibition of oxidation is more severe, so that even with a smaller pool of fatty acids much less of the isotope is recovered. The studies of malnutrition and diabetes suggest that in states of chronic undernutrition or underutilization of carbohydrate the organism adapts by developing the ability to use fat at a more rapid rate.

Growth hormone administration did not increase the rate of fatty acid oxidation as measured by this technique. However, after growth hormone was injected the usual preferential utilization of glucose was blocked. This suggests that the effects of growth hormone to increase catabolism of fat are indirect and secondary to the inhibition of carbohydrate utilization. These studies must be extended and confirmed in order to establish the validity of this hypothesis.

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